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OF TENEBRIO MOLITOR LARVAE

A DISSERTATION SUBMITTED TO THE FACULTY OF
THE DIVISION OF THE BIOLOGICAL SCIENCES IN
CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF ZOÖLOGY
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PATTERNS of morphological change during development have long claimed the attention of biologists. In recent years there has been an added interest in patterns of changing chemical composition during embryonic and larval growth. Various schemata have been designed to establish order in and meaningfulness to the observed data. Apart from simple graphs relating chemical changes to morphological events during development, the most inclusive general system of curves has probably been that which describes heterogonic growth. This concept was first applied on a comprehensive scale by Huxley (1924, 1932) to relative morphological growth and soon thereafter by Teissier (1931) and Needham (1934) to the changing chemical constituents in the growing organism.

Briefly, the family of curves illustrating heterogonic growth, later termed "allometric" by Huxley and Teissier (1936) and "heterauxetic" by Needham and Lerner (1940), is based on the parabola $y = bx^k$, in which y represents the magnitude of the component part, x represents the total organism, and b and k are constants. In logarithmic form this becomes $\log y = \log b + k \log x$. If, when the data are plotted on a double-log grid, the points reasonably fall along a straight line, growth is said to be "heterogonic" or "heterauxetic." The value of k gives the slope of this line and, as such, becomes indicative of whether

the ratio of the rate of growth of the component part is greater than ($k > 1.00$), equal to ($k = 1.00$), or less than ($k < 1.00$) that of the whole. The constant b is less significant, since it merely gives the elevation of the line, or the value of y when x is unity. Needham (1942) recently summarized a large volume of information on the changing chemical patterns during the development of various organisms by expressing the data in terms of k , the constant of relative growth, thereby affording a comparison which widely crosses species lines.

This "differential growth ratio," as Huxley termed it, simply means that empirically an increase of 1 per cent in x is associated with an increase of k per cent in y . Does such a constant change when the organism is subjected to varying external conditions during growth? Is it an expression of a relatively immutable chemical pattern, or does it require a further formulation in order to describe its behavior when systematically varying stresses are imposed on the organism? The present study, which deals with the pattern of lipid composition of the meal worm larva, *Tenebrio molitor* L., raised under different temperatures, was undertaken in an attempt to obtain an answer to these questions.

Chemical heterauxesis was studied extensively in *Tenebrio* by Teissier (1931) but not under differing conditions. He found, for example, that the amount of total fat could be related to the total fresh weight by the expression $y = 0.115 x^{1.13}$. The value of k , 1.13, indicates

¹ The author wishes to thank Dr. W. C. Allee for his interest and encouragement, Dr. Sewall Wright for the privilege of consultation on statistical matters, and Dr. Thomas Park for many helpful suggestions.

that the rate of accumulation of fat occurs more rapidly than that of the total body weight. Similar tachyauxetic values ($k > 1.00$) have also been found for the total fat composition of the waxmoth, rat, and chick (Needham, 1942).

Romanoff and Faber (1933) investigated the growth and fat content of White Leghorn chick embryos raised at five different temperatures from 32° to 40° C. from the 16th day to hatching. Using these data, Needham (1942) calculated the k -values for fat plotted against wet weight and found that, for the four lower temperatures, the values were essentially uniform (1.77–1.84). This study was necessarily limited by the short time interval and hence the small amount of growth, as well as by the narrowness of the range of temperatures tolerated by that organism. A poikilothermic animal, such as the meal worm, offers a wider range of permissible temperatures and, in addition, allows observations over a longer period of time (over 200 days) and over a many-fold increase in weight. Moreover, it is an animal which is very easily reared.

The lipid composition of *Tenebrio* larvae was studied at approximately 20°, 25°, and 30° C. at 25-day intervals. Total lipid, neutral fat, total fatty acids, phospholipid, and cholesterol analyses were made in the course of larval development. The data obtained from these determinations were then analyzed for heterauxetic growth.

MATERIAL AND METHODS

The meal worm larvae were raised in glass dishes measuring 20–24 cm. in diameter and 6 cm. in height, with the culture medium 3–4 cm. deep. This arrangement provided adequate surface for aeration and rapid equilibration of temperature. The food consisted exclu-

sively of bran, which proved to be nutritionally adequate to carry the insects to the adult stage. Waste products, such as excreta, exuviae, and the finely ground "conditioned" bran, were removed, and fresh bran was added at frequent intervals.

Cultures were started by placing an identical number of adults in each of three culture dishes, one for each temperature. The adults were removed after 1 week, and the middle of the week was taken as the starting day for the culture. This procedure involved a small but probably unimportant error, inasmuch as no correction was made for the length of time from egg-laying to hatching. Eggs were laid at the temperatures at which the larvae were maintained.

Culture dishes were placed, uncovered, in incubators, which were kept at roughly 20°, 25°, and 30° C. Table 1 gives the values for the temperature readings in the bran and in the air alongside the dishes. Readings were made almost daily during the period from July 19, 1945, to June 17, 1946, during which period the cultures reported here were raised. The coefficients of variation for these readings ranged from 2.2 to 3.5 per cent and averaged 2.9 per cent. No attempt was made to control the relative humidity, except to insure the absence of very dry conditions by placing a dish of water in each incubator. Approximate relative-humidity readings were made periodically in each of the incubators by means of a dial-type hygrometer. Data derived from these readings are also summarized in Table 1. It is evident that the variations were rather large but that a middle range of humidity was maintained.

At 25-day intervals groups of larvae were removed from each culture, counted, brushed free of adherent particles of medium, and weighed. A sufficient number of larvae were taken to give a fresh

(=wet) weight of at least 0.3 gm. and in most cases of over 1.0 gm. Of the 49 samples, 3 were less than 0.5 gm., and 38 were between 1.0 and 3.0 gm. The number of larvae comprising each sample varied inversely with their size: from 6 to 30 larvae were used when they weighed between 75 and 127 mg., while 100-300 were used when the individual weights ranged from 1.8 to 5.0 mg.

The larvae were placed, after being weighed, in small flasks and covered with

volumetric flask and made up to volume with 95 per cent alcohol. From this extract duplicate determinations were made of total fatty acids, phospholipid, and total and free cholesterol.

Separation of these component lipids was accomplished largely by the procedures outlined by Boyd (1938) and Bloor (1943). Determinations were done by the dichromate-sulphuric acid oxidation method.

Total fatty acids and total cholesterol

TABLE 1
SUMMARY OF TEMPERATURE AND RELATIVE-HUMIDITY DATA

	20° C.		25° C.		30° C.	
	Flour	Air	Flour	Air	Flour	Air
	Temperature					
Mean.....	19.69	19.98	24.48	25.05	30.12	30.16
Standard error.....	0.044	0.038	0.039	0.036	0.060	0.038
Coeff. of var. (%).....	3.50	3.16	2.74	2.38	3.39	2.18
	Relative Humidity (Per Cent)					
Mean.....	63.6		45.9		46.7	
Standard error.....	0.56		0.64		0.67	
Coeff. of var. (%).....	8.0		11.4		13.6	
Vapor tension (mm.).....	11.0		10.8		14.7	
Saturation deficit (mm.)...	6.3		12.7		16.3	

10 ml. of 95 per cent alcohol. Within several days this alcohol was decanted and filtered through fat-free paper (Whatman No. 43) into a 100-ml. volumetric flask. The larvae were then ground with a small amount of sea-sand in a mortar, and the sand-tissue mixture was quantitatively transferred to a thimble of the fat-free filter paper, which was placed in a 200-ml. Underwriter extractor. The material was extracted twice over a hot plate, each time for 15 minutes with fresh 30-ml. portions of a 3:1 alcohol-ether mixture, which was then filtered into the

were first separated by using a 10-25-ml. aliquot, which was pipetted into a 125-ml. Erlenmeyer flask and to which 5 drops of 40 per cent NaOH were added. This was slowly evaporated on a steam bath so that approximately 1 ml. was left after 1 hour. Two to three drops of 0.04 per cent phenol red, followed by 0.2 ml. of 25 per cent sulphuric acid, were added. The mixture was then extracted five or six times with small portions of petroleum ether, which were decanted carefully from the red aqueous portion into a 50-ml. flask and made up

to volume. An aliquot of 2-5 ml. of this was pipetted into a 125-ml. Erlenmeyer flask, evaporated to dryness over steam, gently ventilated with an air stream, and used immediately for oxidation to determine total fatty acids plus total cholesterol. The latter was subtracted, after independent determination, to give a value for total fatty acids alone.

Cholesterol was separated by a combination of the methods of Kelsey (1939) and Boyd (1938). For total cholesterol, aliquots, usually of 20 ml., of the petroleum ether extract were evaporated over steam in flasks down to a few milliliters and were transferred to 15-ml. centrifuge tubes. The flasks were rinsed twice with petroleum ether, and these washings were added to the tubes. The contents of the latter were then gently evaporated, with the aid of boiling-rods, down to $\frac{1}{2}$ ml. in a hot-water bath. Two ml. of acetone were added according to the suggestion of Hunter *et al.* (1945), and the contents were again evaporated to $\frac{1}{2}$ ml. Digtonin, 4 ml. of a 0.2 per cent solution in 95 per cent alcohol, was added and was followed by three drops of 10 per cent hydrochloric acid (Popjak, 1943), and the tubes were stored overnight in the icebox. They were then centrifuged, and the supernatant solvent was discarded. Remaining lipids other than the cholesterol-digtonide precipitate were removed by rinsing twice with petroleum ether, centrifuging, and decanting. From this point on, a modification of Boyd's method was used. The precipitate was dissolved in 5 ml. of absolute methyl alcohol, which was brought to a boil and decanted into the oxidation flasks. This procedure was then repeated with a second 5-ml. portion of methyl alcohol. The combined methanol washings were evaporated over steam, dried with an air stream, and immediately oxidized.

For the separation of the phospholipid

and free-cholesterol fractions, 25-ml. aliquots of the alcohol-ether extracts were placed in 125-ml. Erlenmeyer flasks and evaporated to a few milliliters on a hot plate. The boiling-rod was rinsed with a few drops of petroleum ether and was removed, and the evaporation was completed over steam. A very brief, gentle stream of air was used to blow out any remaining vapors. The residue was immediately extracted three times with small amounts of petroleum ether, which were decanted into a 15-ml. centrifuge tube. In several instances particles of the residue were carried into the centrifuge tubes by the petroleum ether. Such tubes were centrifuged, decanted to fresh tubes, and rinsed with fresh petroleum ether, which was likewise added to the clean tube. After these ether washings were concentrated to $\frac{1}{2}$ ml. by gently boiling the tubes in a hot-water bath, 7 ml. of acetone and 0.1 ml. of 30 per cent magnesium chloride in 95 per cent alcohol were added. The tubes were allowed to stand overnight to obtain a maximum precipitation of the phospholipids. The acetone, containing the free cholesterol, was decanted to fresh tubes, and the residue was rinsed with a small amount of fresh acetone, which, after being centrifuged, was added to the first portion.

The phospholipid was further treated by dissolving the acetone precipitate in 5 ml. of chloroform, which was heated to boiling and poured into an oxidation flask. The tube was rinsed with 2-3 ml. of chloroform, which was brought to boiling and was added to the contents of the oxidation flask. The flasks were evaporated on a steam bath and blown gently with a brief stream of air; the oxidizing agents were added directly.

Free cholesterol was separated by evaporating the phospholipid-free acetone to $\frac{1}{2}$ ml. From this point on, the

samples were treated exactly as were the total cholesterol fractions, i.e., by precipitation with digitonin and three drops of 10 per cent hydrochloric acid.

Oxidation was accomplished by adding 5.0 ml. of Nicloux reagent and 3.0 ml. of approximately N potassium dichromate (Boyd, 1938). The flasks were then placed in an oven at 124°C. for 20 minutes, after which 25 ml. of cold distilled water were added. When the flasks had cooled to room temperature, 10 ml. of freshly prepared 10 per cent potassium iodide were added, and the solutions were titrated with standardized 0.02 N sodium thiosulphate, with 1 per cent starch indicator. Blanks consisting of Nicloux reagent and potassium dichromate were also prepared, heated in the oven with the other flasks, and titrated at this point. Blanks prepared by evaporating comparable portions of the solvents gave results identical with those made up of oxidizing agents alone.

Each item of glassware employed in the extraction, separation, and titration procedures was carefully cleaned before use by means of sulphuric acid-dichromate solution, was thoroughly rinsed in distilled water, and allowed to drain dry. All chemicals used were of reagent grade. Ether was rendered peroxide-free just before use in extraction by shaking it with freshly prepared 10 per cent potassium iodide and rinsing it several times with distilled water.

Results were calculated according to the outline given by Boyd (1938). From the determinations of total fatty acid, phospholipid, and total and free cholesterol, the following were estimated: cholesterol ester fatty acids, phospholipid fatty acids, triglyceride fatty acids, and neutral fat. The total lipid consists of the sum of neutral fat, phospholipid, total cholesterol, and cholesterol fatty acids. The various values for each extract

were then converted to grams per 1,000 larvae (mg/larva).

Duplicate determinations were made, and the median percentage agreement between these ranged from 2.4 per cent for total fatty acids to 11.2 per cent for total cholesterol; the values for free cholesterol and phospholipid were 5.3 and 5.5 per cent, respectively. Statistical evaluation of the results was done according to standard methods, referred to in detail later. *P*-values were derived from tables of "Student's" distribution (1925), and 0.05 was taken as the upper (5 per cent) level of significance.

EXPERIMENTAL RESULTS

GROWTH OF LARVAE

The growth data for the three cultures which were followed most completely are given in Table 2. It will be seen that there is considerable variation among cultures at any one temperature. In order to have some measure of this variability, triplicate samples of equal numbers were weighed for Series III. Lipid analysis was done on one of these samples, and the others were returned to the culture. Since the standard error of a mean is the standard deviation of a sampling distribution of means, the latter statistic was calculated from the means of the three samples. The standard deviation of a single sample of larvae was then estimated by a reversal of the usual calculation, namely, by multiplying the standard error of the mean (calculated as the standard deviation of the three sample means) by $\sqrt{n}$ of the sample. This value and the coefficient of variation obtained by dividing it by the mean are given for each of the several age and temperature levels in the second part of the table. That there is a marked variation among cultures is not surprising, in view of the variation from sample to sample within a single culture.

TABLE 2
GROWTH OF LARVAE AT DIFFERENT TEMPERATURES

DAYS	WEIGHT (MG.) PER LARVA							
	Culture I (Mean)	Culture II (Mean)	Culture III (Mean)	Total (Mean)	Variation in Culture III			
					No.	Standard Error	Standard Deviation	Coeff. of Variation (%)
20° C.								
50.....	2.04		1.86	1.95	100	0.0596	0.596	32.04
75.....	4.61	6.47	5.19	5.42	50	0.1556	1.100	21.20
100.....	15.53	22.82		19.18				
125.....	48.34	40.70		44.52				
150.....	94.74	82.95	92.21	89.97	7	6.5079	17.218	18.67
175.....		108.72		108.72				
200.....			127.03	127.03				
Mean.....								23.97
25° C.								
50.....	3.04	3.46	2.78	3.09	100	0.1046	1.046	37.67
75.....	9.76	12.42	8.40	10.19	50	1.2720	8.994	107.10
100.....	30.73	36.20	21.52	29.48	20	1.3497	6.036	28.05
125.....	57.15	49.61		53.38				
150.....	91.57	81.20	49.74	74.17	20	0.4781	2.138	4.30
175.....	95.71	92.61		94.16				
200.....	100.63		89.08	94.86	12	2.4414	8.457	9.66
220.....			110.81		20	6.3640	28.461	24.66
225.....		84.56		97.69				
250.....		93.52		93.52				
Mean.....								35.24
30° C.								
50.....	7.21	8.22	7.54	7.66	75	0.3022	2.617	34.68
75.....	25.51	24.43	16.16	22.03	40	1.2076	7.638	47.28
100.....	50.92	34.99	16.76	34.22	20	1.9277	8.621	51.45
125.....	51.84	39.12		45.48				
150.....	59.64	58.29	49.29	55.74	20	2.9167	13.044	26.46
175.....	64.84	53.11		58.98				
200.....	63.73			63.73				
225.....		44.17		44.17				
250.....	49.12	40.91		45.02				
275.....	44.52	53.94		49.23				
300.....	48.01			48.01				
Mean.....								39.97

ERRATUM

Part of the legend for Figure 2 in the article entitled "The Lipid Composition of *Tenebrio molitor* Larvae," by Asher J. Finkel, of the Department of Zoölogy, University of Chicago, was inadvertently omitted in the April, 1948, issue of *Physiological Zoölogy* (p. 117). It is given in full herewith.

FIG. 2.—Growth of meal-worm larvae at different temperatures and humidities

	Source	Temperature (° C.)	Rel. Hum. (Per Cent)
Upper	A.....	31.1	80
	B.....	31.1	50
	C.....	30.0	46
Middle	A.....	24.7	80
	B.....	24.7	50
	C.....	25.1	46
Lower	A.....	21.1	80
	B.....	21.1	50
	C.....	20.0	64

The mean growth curves of the larvae at each of the three temperatures are plotted in Figure 1. It is apparent that the larvae at the lower temperatures grew faster, after a slow start, and reached pupation earlier than did those at the higher temperatures. These curious results are undoubtedly referable to the differences in relative humidity, for

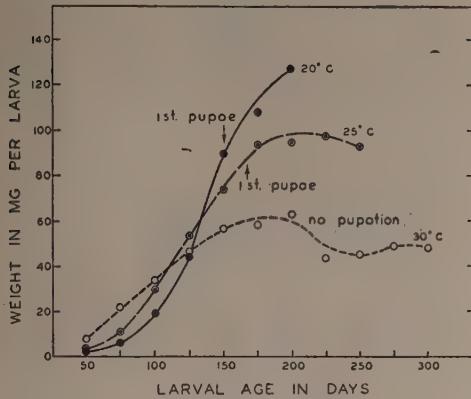


FIG. 1.—Growth of meal-worm larvae at different temperatures.

interpolation of these curves with the data given by Menusan (1936) gives a rather consistent picture (Fig. 2). It is quite evident that humidity is at least as important as is temperature in influencing the larval growth of this organism.

LIPID COMPOSITION OF LARVAE

Data on lipid composition are presented in Table 3 for each of the three series which were analyzed in detail. These constitute the basic information of this paper. Data have been restricted to 200 days, since either pupation was well under way at this time (20°, 25° C.) or a maximum in the growth curve had been reached (25°, 30° C.). For each value in the table the percentage of the wet weight was calculated, and the means of these have been included in the table.

Apart from occasional variations, there is a general consistency in the trends shown by the results. As larval growth progresses, the total lipid becomes an increasing proportion of the body weight (Table 3, A). Neutral fat, as the major component of the total lipid, similarly increases with respect to total weight, as do the total fatty acids (Table 3, B, C). Total cholesterol, on the other hand, while it is increased in amount with larval growth, maintains a nearly constant proportion to the total weight. The mean per cent wet-weight values for this component vary only be-

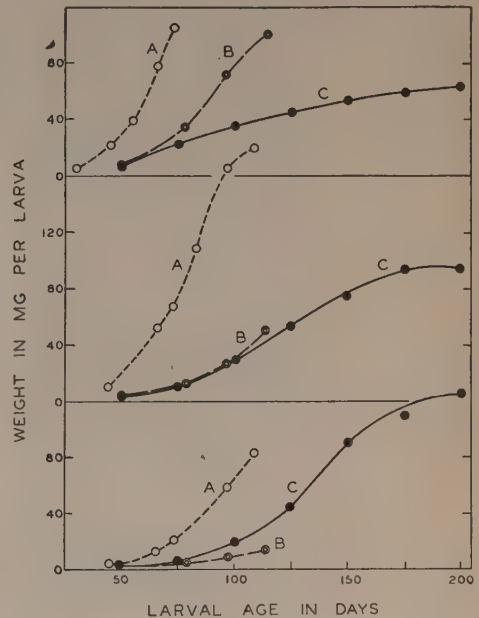


FIG. 2.—Growth of meal-worm larvae at different temperatures and humidities.

tween 0.11 and 0.32 per cent, except at 175 days (Table 3, D). The picture with this component is complicated by the unusually large values at all temperatures in Series III at 150 days and in Series II at 175 days. These discrepancies are traceable to departures from the procedures for separation of total cholesterol which occurred only in these in-

TABLE 3

LIPID COMPOSITION OF MEAL WORMS IN GRAMS PER 1,000 LARVAE

AGE IN DAYS	20° C.				25° C.				30° C.				
	Culture			Mean Per Cent of Wet Weight	Culture			Mean Per Cent of Wet Weight	Culture			Mean Per Cent of Wet Weight	
	I	II	III		I	II	III		I	II	III		
	A. Total Lipid												
	50.....	0.14	0.18	8.36	0.24	0.24	7.79	0.68	0.74	9.05			
	75.....	0.49	0.47	0.49	9.10	1.04	1.14	0.84	10.01	2.96	2.54	1.62	10.69
	100.....	1.44	2.30	9.65	3.39	4.38	2.54	11.65	6.87	4.92	2.12	13.41	
	125.....	5.83	4.82	11.95	8.01	7.22	14.28	8.42	5.91	15.67			
	150.....	11.73	9.66	11.84	12.29	14.43	12.10	6.07	14.29	10.59	7.01	3.03	11.97
	175.....	11.22	10.32	13.64	14.73	6.11	11.49						
	200.....	16.38	12.90	15.02	16.69	17.24	8.37	13.13					
	B. Neutral Fat												
	50.....	0.09	0.14	5.86	0.16	0.18	5.51	0.53	0.62	7.79			
	75.....	0.35	0.30	0.39	6.57	0.73	0.80	0.72	7.48	2.26	1.87	1.42	8.44
	100.....	1.07	1.77	7.32	2.67	3.63	2.21	9.67	5.77	4.12	1.86	11.39	
	125.....	5.23	3.86	10.16	7.34	6.20	12.67	7.83	5.00	13.94			
	150.....	10.47	8.52	10.43	10.88	13.37	11.31	5.46	13.17	9.71	6.32	2.33	10.62
	175.....	9.38	8.63	11.99	12.95	5.34	10.06						
	200.....	14.82	11.66	13.83	16.01	16.25	7.88	12.36					
	C. Total Fatty Acids												
	50.....	0.12	0.16	7.11	0.20	0.21	6.63	0.59	0.66	7.98			
	75.....	0.41	0.39	0.43	7.74	0.88	0.98	0.76	8.62	2.54	2.20	1.47	9.35
	100.....	1.24	2.00	8.38	2.98	3.90	2.30	10.38	6.16	4.41	1.92	12.04	
125.....	5.31	4.27	10.74	7.36	6.53	13.02	7.78	5.32	14.31				
150.....	10.68	8.77	10.64	11.13	13.33	11.19	5.44	13.09	9.75	6.40	2.54	10.83	
175.....	9.89	9.09	12.21	13.19	5.44	10.24							
200.....	15.01	11.82	13.86	15.58	16.02	7.77	12.18						
D. Total Cholesterol													
50.....	0.01	0.00	0.23	0.01	0.00	0.22	0.02	0.01	0.19				
75.....	0.02	0.01	0.01	0.30	0.05	0.02	0.02	0.31	0.14	0.04	0.03	0.30	
100.....	0.04	0.05	0.24	0.07	0.09	0.05	0.24	0.11	0.07	0.04	0.22		
125.....	0.11	0.06	0.18	0.10	0.07	0.16	0.10	0.05	0.17				
150.....	0.20	0.16	0.43	0.29	0.13	0.15	0.32	0.32	0.09	0.12	0.28	0.31	
175.....	0.51	0.47	0.58	0.63	0.30	0.57							
200.....	0.21	0.17	0.11	0.15	0.14	0.07	0.11						
E. Phospholipid													
50.....	0.04	0.04	2.23	0.07	0.05	2.06	0.14	0.11	1.54				
75.....	0.11	0.16	0.09	2.16	0.24	0.32	0.11	2.11	0.49	0.62	0.17	1.84	
100.....	0.32	0.47	2.05	0.62	0.64	0.27	1.69	0.96	0.73	0.22	1.75		
125.....	0.46	0.90	1.58	0.54	0.94	1.73	0.45	0.86	1.53				
150.....	0.99	0.94	1.00	0.89	0.59	0.13	0.65	0.77	0.74	0.26	0.91		
175.....	1.08	0.99	0.74	0.80	0.30	0.56							
200.....	1.26	0.99	1.06	0.48	0.81	0.42	0.66						

stances. Probable values can be estimated from the free-cholesterol determinations, which were not affected. For each temperature the mean per cent that free cholesterol constituted of the total cholesterol, with its standard deviation, follows: for 20°, 56.4 ± 8.9 per cent; for 25°, 64.7 ± 5.0 per cent; for 30°, 66.4 ± 6.2 per cent. When these percentages are used to estimate values of the total cholesterol, more consistent results are obtained, as shown in the accompanying tabulation. Phospholipid,

Days	20°	25°	30°
150.....	0.20%	0.17%	0.17%
175.....	0.22%	0.17%	0.15%

in contrast to neutral fat, declines very slowly in percentage, so that at 200 days it forms less than 1 per cent of the total weight, while at 50 days it constitutes from 1.5 to 2.2 per cent (Table 3, E).

PERCENTAGE COMPOSITION OF TOTAL LIPIDS

These relations are made more striking by a consideration of the changing composition of the lipids as such. When the values are calculated in terms of percentage of total lipid, the trends shown in Table 4 are seen. Neutral fat becomes an increasingly greater component of the total lipid as larval development progresses. Phospholipids, contrariwise, show a fairly steady decline in this regard, although, as has been seen, they maintain a small but almost constant percentage of the wet weight of the organism. The total fatty acids combine both these trends by a slowly but consistently increasing share of the total lipid composition. Total cholesterol shows practically no change in its proportion to total fat; the values at 150 and 175 days, as has been explained, are probably incorrectly large. In Figure 3,

in which the various components are plotted, the estimated values based on the free-cholesterol determinations have been used.

HETERAUXESIS

The data were next analyzed for allometry. Figure 4 shows the double-log plot of total lipids against wet weight for each of the temperatures. Since the points show a fairly good distribution along a straight line, they may be said to behave heterauxetically. Values of k were calculated by fitting a least-squares straight line to the logarithms of the weights. The regression coefficient of such a line is identical with the relative growth constant of the heterogonic growth formula. This calculation was done for each culture series at each temperature. The results are given in Table 5, which also includes the standard error of estimate ($\bar{S}_{y \cdot x}$), the standard error of the regression coefficient (σ_k), and a P -value. The standard error of estimate is calculated from the difference between the observed values and those given by the best-fitting straight line and is adjusted for the number of points to give an unbiased estimate (Ezekiel, 1941). It is thus a measure of the closeness of fit of the points to the line drawn through them. The standard error of k is a measure of the variability of the slope as estimated from the sample at hand. It is interpreted in the same manner as is the standard error of a mean. The P -value given in Table 5 is derived from the t -test of significance, which evaluates the difference between the observed k -value and a theoretical value (Smith, 1939). In this instance a theoretical value of 1.00 was selected because it represents a rate of growth identical with that of the total. The P -values, obtained from "Student"'s distribution for small numbers, give a measure of the significance of the departures

of the observed values of k from the isauxetic value of 1.00. These statistical indices were then calculated for the combination of the three series for each temperature to give over-all values at each of the temperatures and again for all the data combined.

Of the nine elements in Table 5, where the series and temperatures are considered individually, five show a statistically significant increase in the k -values over 1.00, and these instances are all at 20° and 25° C. When the three series are combined at each temperature, the values of k range from 1.098 at 20°

to 1.202 at 25°, each of which departs significantly from 1.00. However, there is an intermediate value of 1.165 at 30°, which is not significant because of the greater scatter of the points about this line. The greater variation at 30° is indicated by the higher value of $\bar{S}_{y-x}$ at this temperature. The results of the t -tests for significance of the differences between the k -values of the several temperatures are given in Table 6, A, where it can be seen that only the 20° versus 25° comparison yields a significant difference.

The values of k have been subjected to a further evaluation by an analysis

TABLE 4
LIPID DISTRIBUTION IN MEAN PERCENTAGE OF TOTAL LIPID

DAYS	NEUTRAL FAT	PHOSPHO- LIPID	TOTAL CHOLESTEROL	CHOLESTEROL ESTER FATTY ACID	TOTAL LIPID	TOTAL FATTY ACIDS
20° C.						
50.....	68.89	27.61	2.85	0.65	100.00	84.90
75.....	71.50	24.49	3.31	0.69	99.99	84.97
100.....	75.78	21.23	2.46	0.54	100.01	86.76
125.....	84.92	13.29	1.52	0.28	100.01	89.86
150.....	88.51	8.22	2.31	0.95	99.99	90.55
175.....	83.61	9.62	4.54	2.24	100.01	88.10
200.....	90.44	7.71	1.31	0.54	100.00	91.63
25° C.						
50.....	70.14	26.82	2.77	0.27	100.00	84.82
75.....	74.75	21.27	3.05	0.94	100.01	86.14
100.....	82.82	14.60	2.05	0.53	100.00	88.99
125.....	88.78	9.94	1.12	0.17	100.01	90.87
150.....	92.02	4.38	2.47	1.12	99.99	91.48
175.....	87.95	5.42	4.26	2.36	99.99	89.54
200.....	94.04	4.97	0.82	0.18	100.01	92.85
30° C.						
50.....	80.28	17.20	2.15	0.38	100.01	88.14
75.....	79.19	17.15	2.70	0.95	99.99	87.63
100.....	85.01	13.00	1.64	0.35	100.00	89.82
125.....	88.81	9.95	1.05	0.19	100.00	91.23
150.....	86.27	7.84	3.94	1.93	99.98	89.15
175.....	87.46	4.83	4.95	2.74	99.98	89.08
200.....	94.12	5.00	0.84	0.02	99.98	92.79

of variance by which the variation among cultures and among temperatures are tested together. This can be done, since the numbers of observations in each cell are approximately equal. The formulae given by Goulden (1939) were used for this purpose, and the results are given in Table 7, A. The F -values so obtained are far below that for the 5 per cent level

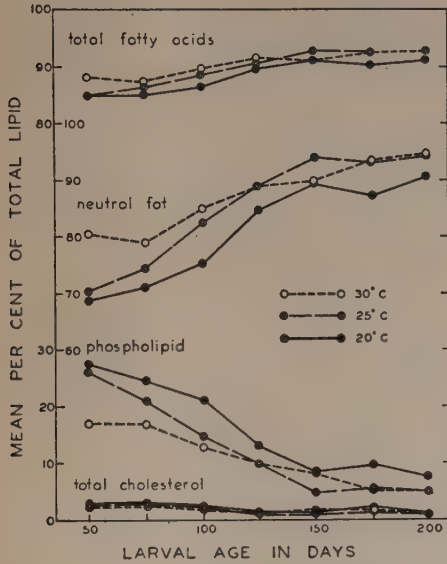


FIG. 3.—Changes with age of mean percentage of total lipid of various components.

of significance (6.94) and hence may be taken to show no significant variation among temperatures or among experiments.

Teissier reported a k of 1.13 for his data of total fat against total wet weight. Recalculation from his published data by the least-squares method used here gives a value of 1.117 with a standard error (σ_k) of 0.024. This standard error is the same as that obtained by grouping all the data in the present paper, for which k is 1.149. The good agreement between these two k -values is shown by the lack of a statistically significant difference, since the t -test gives a ratio of 0.93.

Similar analyses can be made for the several lipid components in relation to the total lipid. Its major constituent, neutral fat, shows good heterauxesis with respect to the total (Fig. 5). The data are summarized in Table 8, A. That these values fit a straight line even more closely than do those of the total lipid against wet weight is indicated by their smaller standard error of estimate. The k -values for each of the temperatures, although very close to 1.00, also depart signifi-

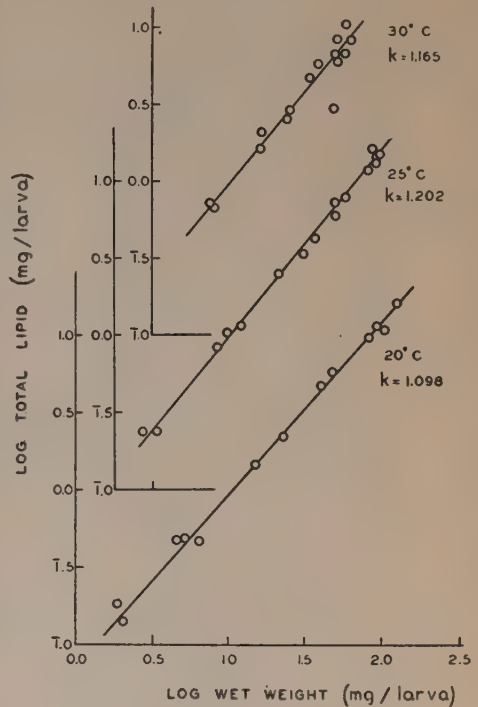


FIG. 4.—Double-log plot of total lipid against wet weight at various temperatures.

cantly from isauxesis, and this situation is especially true for the two lower temperatures. None of the comparisons between temperatures shows a significant difference by the t -test (Table 6, B). Analysis of variance (Table 7, B) reveals practically no differences among temperatures and quite unimportant differences among experiments.

TABLE 5
HETERAUXETIC CONSTANTS FOR TOTAL LIPID
IN RELATION TO WET WEIGHT

SERIES	HETERAUX. CONSTANT k	S.E. OF ESTIMATE $\bar{S}_{y \cdot x}$	S.E. OF REGRESS. COEFF. σ_k	P	n
20° C.					
I.	1.070	0.052	0.052	0.3152	4
II.	1.136	0.049	0.036	0.0197	6
III.	1.078	0.026	0.017	0.0421	4
Combined.	1.098	0.057	0.024	0.0015	14
25° C.					
I.	1.170	0.035	0.042	0.0273	5
II.	1.240	0.023	0.019	0.0000	6
III.	1.202	0.067	0.056	0.0365	5
Combined.	1.202	0.045	0.023	0.0002	16
30° C.					
I.	1.300	0.066	0.207	0.2458	5
II.	1.221	0.070	0.100	0.0926	6
III.	0.724	0.116	0.200	0.3044	4
Combined.	1.165	0.110	0.095	0.1058	15
Total data.	1.149	0.080	0.024	0.0000	45
Teissier's data.	1.117	0.033	0.024	0.0006	12

TABLE 6
COMPARISONS OF HETERAUXETIC CONSTANTS

	20° vs. 25°		20° vs. 30°		25° vs. 30°	
	t	P	t	P	t	P
A. $\frac{\text{Total lipid}}{\text{Wet weight}}$	3.15	0.0040	0.69	Not signif.	0.38	Not signif.
B. $\frac{\text{Neutral fat}}{\text{Total lipid}}$	0.60	Not signif.	0.50	Not signif.	0.92	Not signif.
C. $\frac{\text{Phospholipid}}{\text{Total lipid}}$	1.39	Not signif.	0.43	Not signif.	0.54	Not signif.
D. $\frac{\text{Total cholesterol}}{\text{Total lipid}}$	1.23	Not signif.	0.74	Not signif.	0.20	Not signif.
E. $\frac{\text{Total fatty acid}}{\text{Total lipid}}$	0.41	Not signif.	0.11	Not signif.	0.36	Not signif.

The decline in percentage composition of phospholipid with respect to the total lipid as development proceeds is reflected in the mean k -values, which range from 0.559 to 0.704 for the several tem-

peratures (Table 8, B). That there is considerable scatter of points about the straight lines can be seen in Figure 6 and from the $\bar{S}_{y \cdot x}$ values, which are larger than those in the two categories already

TABLE 7
ANALYSIS OF VARIANCE

	SUM OF SQUARES	DEGREES OF FREEDOM	VARIANCE σ^2	F^*
A. Total Lipid versus Wet Weight				
Total.....	0.2273	8		
Between temperatures.....	0.0390	2	0.0195	0.67
Between series.....	0.0712	2	0.0356	1.22
Interaction.....	0.1171	4	0.0293	
B. Neutral Fat versus Total Lipid				
Total.....	0.0241	8		
Between temperatures.....	0.0006	2	0.0003	0.14
Between series.....	0.0154	2	0.0077	3.79
Interaction.....	0.0081	4	0.0020	
C. Phospholipid versus Total Lipid				
Total.....	0.2511	8		
Between temperatures.....	0.0948	2	0.0474	2.24
Between series.....	0.0718	2	0.0359	1.70
Interaction.....	0.0845	4	0.0211	
D. Total Cholesterol versus Total Lipid				
Total.....	1.5842	8		
Between temperatures.....	0.1494	2	0.0747	0.54
Between series.....	0.8907	2	0.4454	3.27
Interaction.....	0.5441	4	0.1360	
E. Total Fatty Acid versus Total Lipid				
Total.....	0.0046	8		
Between temperatures.....	0.0000	2	0.0000	0.00
Between series.....	0.0027	2	0.0014	2.78
Interaction.....	0.0019	4	0.0005	

* For comparisons of variance of temperature and of series with interaction, the F -values for the 5 and 1 per cent levels of significance, respectively, are 6.94 and 18.00.

discussed. In spite of this variation the *P*-values indicate that the heterauxetic constants again depart significantly from 1.00, especially at the lower temperatures. Here, too, the differences between temperatures are not significant by direct comparison (Table 6, C) or by the analysis of variance (Table 7, C).

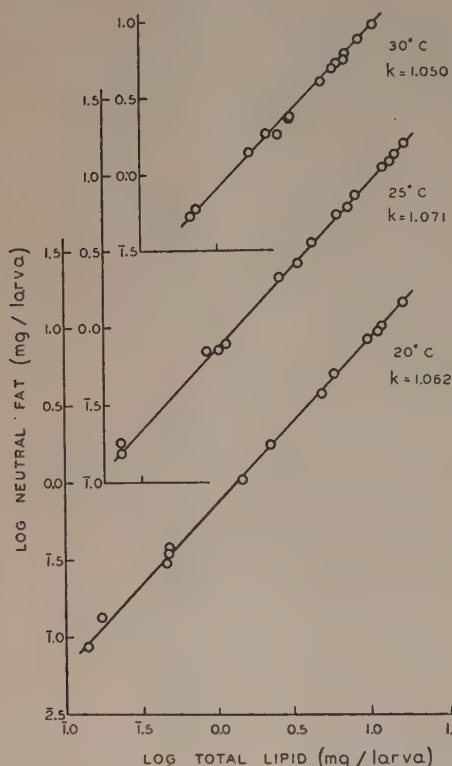


FIG. 5.—Double-log plot of neutral fat against total lipid at various temperatures.

Teissier (1931) reported a *k* of 0.83 for lipid phosphorus against wet weight. Calculation of *k* by least squares from his data gives a value of 0.844, with a standard error of estimate of 0.029 and a σ_k of 0.024. Since lipid phosphorus constitutes an almost constant fraction (approximately 1/26) of phospholipid, the relative rates of increase of these two substances should be identical when measured simultaneously. In the present

investigation the over-all value of *k*, for all temperatures combined, for phospholipid against total lipid is 0.628. This can be converted to the value for phospholipid versus wet weight by multiplying by the value for total lipid versus wet weight, viz., $0.628 \times 1.149 = 0.722$. The latter value is somewhat lower than that found by Teissier but is roughly similar to it.

The data for total cholesterol show a fit to rectilinearity comparable to that for phospholipid, since the standard errors of estimate for these two components are of the same magnitude. In calculating the regression coefficients for total cholesterol, the values of Series III at 150 days and of Series II at 175 days have been omitted. Their wide divergence from the bulk of the data can be appreciated from Figure 7, where they are indicated by closed circles; and the inclusion of these data in the calculations produces standard errors of estimate which are quite large. The *k*-values given in Table 8, C, are less than 1.00, and they depart from it significantly at the lower temperatures and almost so at 30°. They do not differ from one another significantly (Table 6, D). Analysis of variance reveals no significant differences among temperatures or among series.

The final component analyzed by these methods was that of total fatty acids, which comprise 95 per cent of the neutral fat and 67 per cent of the phospholipid, plus the small amount of cholesterol-ester fatty acids. When the total fatty acids are plotted against the total lipid, the points show an exceedingly good fit to a straight line (Fig. 8), and calculations demonstrate a consistent and unusually small standard error of estimate at each of the temperatures (Table 8, D). Although the slope of the line at each temperature is quite close to 1.00, it departs from it very significantly at the

TABLE 8
HETEROAUXETIC CONSTANTS FOR COMPONENTS OF TOTAL LIPID

	TEMPERATURE						GRAND TOTAL	
	20° C.		25° C.		30° C.			
	<i>n</i>	<i>k</i>	<i>n</i>	<i>k</i>	<i>n</i>	<i>k</i>	<i>n</i>	<i>k</i>
A. Neutral Fat versus Total Lipid								
I.....	4	1.079	5	1.109	5	1.168		
II.....	6	1.083	6	1.089	6	1.066		
III.....	4	1.036	5	1.053	4	0.966		
Total.....	14	1.062	16	1.071	15	1.050	45	1.066
$\bar{S}_{y \cdot x}$		0.030		0.028		0.028		
σ_k		0.011		0.012		0.020		
<i>P</i>		0.0002		0.0002		0.0271		
B. Phospholipid versus Total Lipid								
I.....	4	0.624	5	0.479	5	0.149		
II.....	6	0.707	6	0.553	6	0.569		
III.....	4	0.733	5	0.437	4	0.642		
Total.....	14	0.704	16	0.559	15	0.648	45	0.628
$\bar{S}_{y \cdot x}$		0.122		0.225		0.183		
σ_k		0.046		0.094		0.130		
<i>P</i>		0.0002		0.0004		0.0182		
C. Total Cholesterol versus Total Lipid*								
I.....	4	0.707	5	0.325	5	-0.411		
II.....	5	0.740	5	0.698	5	0.694		
III.....	3	0.916	4	0.842	3	1.133		
Total.....	12	0.824	14	0.713	13	0.716	39	0.752
$\bar{S}_{y \cdot x}$		0.136		0.161		0.183		
σ_k		0.056		0.070		0.132		
<i>P</i>		0.0108		0.0014		0.0558		
D. Total Fatty Acids versus Total Lipid								
I.....	4	1.028	5	1.027	5	1.063		
II.....	6	1.021	6	1.025	6	1.019		
III.....	4	1.010	5	1.014	4	0.970		
Total.....	14	1.017	16	1.020	15	1.016	45	1.019
$\bar{S}_{y \cdot x}$		0.010		0.009		0.011		
σ_k		0.004		0.004		0.008		
<i>P</i>		0.0004		0.0002		0.0533		

* The omission of data of Series II at 150 days and of Series III at 175 days is explained in the text.

two lower temperatures and almost so at 30°. Direct comparisons indicate no significant differences between the k -values for the three temperatures (Table 6, E). The analysis of variance supports this conclusion (Table 7, E), for whatever variation is present is seen to reside principally among experiments.

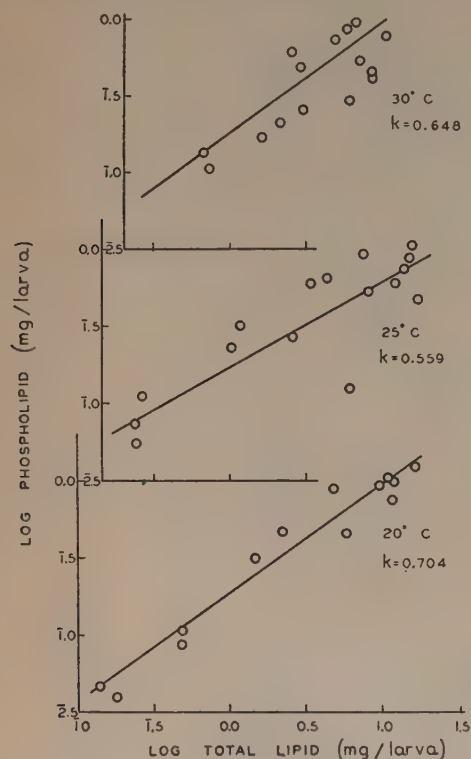


FIG. 6.—Double-log plot of phospholipid against total lipid at various temperatures.

DISCUSSION

The data presented demonstrate that, among *Tenebrio* larvae, there is an appreciable degree of variation in growth, even when the organisms are grown under identical conditions of temperature and humidity. These findings with respect to variability are consistent with the experience of other investigators working with this animal, e.g., Cotton (1927). As is evident from Table 2, not

only is there variation from culture to culture, but considerable differences are also present among the individuals within a culture. It is doubtful that lack of food or the accumulation of waste products was responsible for the variation, since the bran in which the larvae developed was changed frequently enough to provide an ample supply of fresh medium.

In addition to the general tendency toward variability, there seems to be

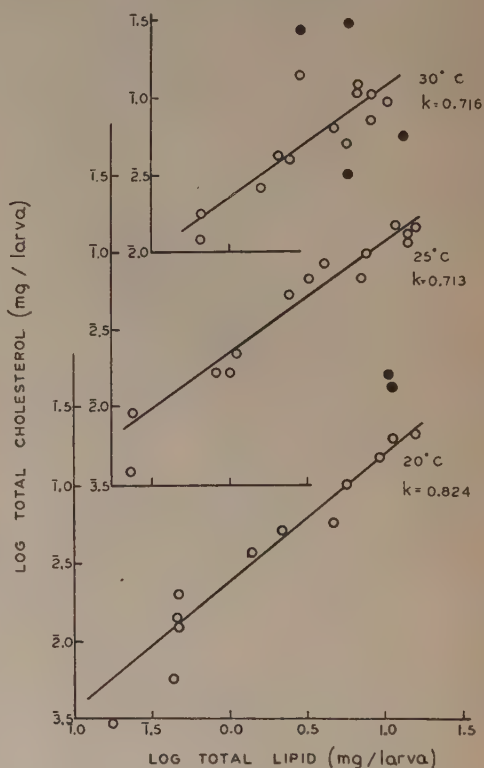


FIG. 7.—Double-log plot of total cholesterol against total lipid at various temperatures.

some relation between temperature and the degree of variation, at least over the range studied. The mean coefficients of variation for weights within a culture (Table 2) show a progressive increase with higher temperature levels. This situation is in contrast with that of the rela-

tive growth constants of the lipid components, for not only is the extent of variation of these of the same order of magnitude from temperature to temperature within each category of lipid (Tables 5 and 8), but there is at least an equal degree of consistency between temperatures as there is between experiments run at each temperature (Table 7).

The differences among the growth curves of the larvae at the three temperatures studied (Fig. 1) probably can be accounted for by certain well-established principles: (1) Except at the extremes of the tolerated temperature range, poikilothermic organisms tend to develop more rapidly at higher temperatures. This needs no elaboration, having been well established for insects (Uvarov, 1931) as well as for other organisms. For *T. molitor*, in particular, Lengerken (1925) reported that the length of larval life was increased by many months and that supernumerary molts occurred when these insects were kept in a cool cellar. Singh-Pruthi (1924) also showed that many more molts occur in these forms at 23° and 26.5° than at 29.5° C. (2) Many animals, although they develop more slowly, tend to reach a larger size when they are raised at lower temperatures. Titschak (1925) showed that larger and heavier clothes moths (*Tineola biselliella*) were produced at lower temperatures. He accounted for these results by the increase in the length of larval life and in the total amount of food ingested, by the greater percentage of food absorbed, and by the greater utilization of food for the building of body tissues at colder temperatures. Uvarov (1931) cites other instances of larger size at lower temperatures in insects, while Hesse, Allee, and Schmidt (1937) refer to many similar instances among marine organisms. (3) Many insects living on stored products grow faster when the relative humidity

is higher (Ahmad, 1936; Fraenkel and Blewett, 1943). In a further study in 1944 the latter authors demonstrated that *Tribolium confusum*, *Ephestia kuhniella*, and *Dermestes vulpinus* ate more food under drier conditions to produce a given unit of body weight, while the moisture content of the pupae remained practically constant. Under these circum-

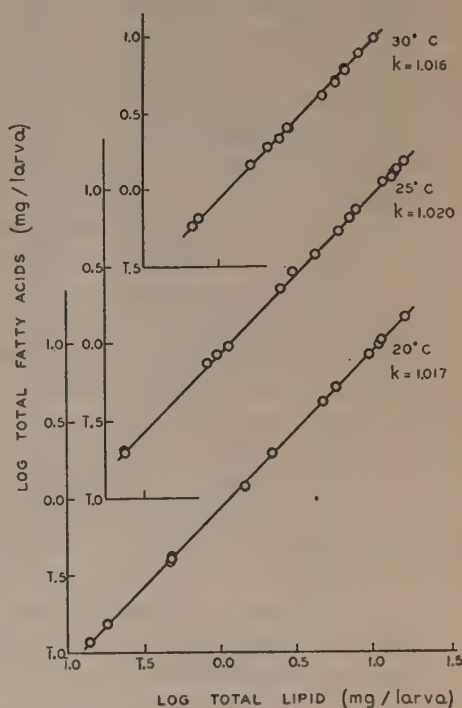


FIG. 8.—Double-log plot of total fatty acids against total lipid at various temperatures.

stances the length of the larval period increased, the larvae grew more slowly, and the weight of the pupae decreased. In this connection, Evans and Goodliffe (1939) showed that the feeding larvae of *Tenebrio* obtain practically no water from ingested bran, but they calculated that metabolic water is produced from the combustion of carbohydrates. Since, in the present experiments, the highest value for relative humidity was associated with the lowest temperature, it is

not surprising that this curve of larval growth was heightened and the duration of larval life shortened by virtue of the effect of moisture. This conclusion is further strengthened by the consistent picture obtained when the present data are plotted together with those of Menusan (1936) in Figure 2.

Another effect of high temperature may be of additional value in explaining the growth curves. The late larval development and the metamorphosis of meal worms is known to be delayed when these animals are subjected to temperatures of 30° C. (Singh-Pruthi, 1924). This finding may explain the drop, after 200 days, in the curve at 30° in Figure 1 and the almost complete failure of these cultures to produce pupae.

In spite of the dependence of growth on temperature and humidity, earlier work indicates that the moisture content of the larvae is largely unaffected by these factors. Meal worms have long been known to maintain an almost unchanging ratio of body water under dry conditions (Berger, 1907). Buxton (1930) showed that at 23° C. the proportion of dry matter to water is constant at various humidities in fasting *Tenebrio* larvae. Whether this is accomplished by changing rates of metabolism at different humidities (Buxton) or by altered rates of evaporation (Mellanby, 1932, 1934, 1936), the important point here is that meal worms are capable of maintaining a constant moisture content under a variety of external circumstances (except under hot, moist conditions). Mellanby (1932) suggested that normal meal worms lose water at a rate proportional to the saturation deficit when the relative humidity is less than 88 per cent. He pointed out that, because of their marked ability to conserve water, *Tenebrionid* beetles are adapted to live in hot, dry air or in cooler air at various humidities.

In view of the fact that many physiological processes among insects are dependent upon the relative humidity, while many others are correlated instead with the saturation deficiency (Ludwig, 1945), it was considered to be sufficient in the present work to maintain the cultures at fairly constant humidity levels in the middle of the tolerated moisture range. The differences in the resulting curves of growth fortified the validity of the conclusions concerning the heterauxetic constants.

Previous studies of meal worm lipids have consisted largely in charting the changes during pupation. Becker (1934) found that larval and pupal fats were practically indistinguishable qualitatively and that young adult fat was very similar, except for an increase in nonsaponifiable matter (4.4 per cent against 0.93 for pupae and 1.56 for larvae). This trend became even more marked in old adults, in which the unsaponifiable matter reached 6.3 per cent. In addition to these qualitative changes, he found that the percentage of fat declined with metamorphosis, being 12–14 per cent in larvae, 8.9 in pupae, 8.1 in young, and 4.6 in old adults. On the other hand, Evans (1934) found that the amount of unsaponifiable material, as well as the amount of fatty acids, remained constant during the transformation from larva to adult.

The present study was concerned with an earlier phase of meal worm development. Here, in the long larval period, definite trends in the changing lipid composition appeared. Total lipid was found to become an increasing proportion of body weight, ranging from 8–9 per cent of the fresh weight at 50 days to 12–17 per cent at pupation or at 200 days. The latter range is consistent with Becker's findings. It is not surprising that such an increase should occur, for the larval pe-

riod is commonly thought to be a specialized portion of the life-cycle devoted to food-gathering and deposition of nutritional reserves. In insects this larval role is emphasized by the presence of a storage organ, the fat body, which, Wigglesworth (1939) points out, is most conspicuous in the larvae of holometabolous forms. The fat content of certain insects, silkworms in particular, reaches rather high values (Uvarov, 1928). Rudolfs (1926) followed the fat content in the tent caterpillar throughout development and found that during the period from the freshly hatched larva to pupation the ether-soluble extract in percentage of dry weight rose from about 1 to 29 per cent. Kemnitz (1916), with the botfly, *Gastrophilus equi*, found a steady increase of ether extractives from 3.54 to 26 per cent on a dry-weight basis (corresponding to 0.8-9.0 per cent of fresh weight) in larvae ranging in fresh weight from 0.2 to 0.515 gm. In the flesh fly, *Lucilia sericata*, total ether extractables were found by Yuill and Craig (1937) to increase from 6 per cent in young larvae to 30 per cent in fully grown ones. Melampy *et al.* (1940) reported increases in the total lipid in the larvae of queen and worker honeybees, with a maximum at or shortly before pupation. Similar results had been obtained by Straus (1911) for worker and drone bees.

What gives these data on insects larger significance is the fact that they are consistent with trends widely found throughout the animal kingdom. An increase in the percentage of fat with growth has been found in chick embryos (Murray, 1926; Romanoff, 1932, and others cited by Needham, 1931), in rabbit fetuses (Fehling, 1877), and in human fetuses, (Fehling, 1877; Aron, 1913; Iob and Swanson, 1934). A slight rise in the absolute amount of fat as well as its percentage of the dry weight was found by Gortner

(1914) and by McClendon (1915) in growing salamander eggs, while Needham (1931) cites the work of Burdach in 1853, who found an increase in neutral fat in the egg of the snail, *Lymnaea stagnalis*, during development. This rise does not represent a universal phenomenon, however, for Gortner (1945) found that in pig embryos the total lipid percentage of the wet weight remained fairly constant at about 1 per cent.

In addition, mammals show a striking increase in fat content with age in the postnatal period (Moulton, 1923). This generalization is supported by the work of Chanutin (1931) and more recently of Williams *et al.* (1945) on rats. The importance of this trend is attested to by the long-standing practice of reducing data on chemical composition of tissues to a fat-free, as well as a moisture-free, basis (Aron, 1913). Parallel with the increase in total fat is the well-established decrease in the relative water content (Moulton, 1923; Needham, 1942).

The increase in the relative fat content which occurs during growth is not a simple measure of the storage of reserve materials. After the work of Mayer and Schaffer and of Terroine it has been recognized that fatty substances in tissues can be divided into two great categories: the *élément constant*, consisting of the phospholipids (especially of lecithin) and of cholesterol, and the *élément variable*, comprising the neutral fats (Bloor, 1945). The latter represents the storage deposits, while the former group of substances is essential to and characterizes tissue structures and activities. The lipids of the meal worm show a generally increasing proportion of neutral fat, reaching 90-94 per cent of the total lipid by 200 days, reflecting the accumulation of reserves preceding pupation. This trend is mirrored by the generally decreasing percentage of essential lipids

(phospholipid and cholesterol). Young rats show similar trends in the percentage lipid distribution, with neutral fat rising from 55 per cent at birth to 87 per cent at 70 days, while there is a concomitant downward trend for essential lipids (Williams *et al.*, 1945).

In terms of its relation to total fresh weight, the phospholipid of meal worm larvae was seen to constitute an almost constant proportion, declining slowly from 1.5–2.2 per cent at 50 days to 0.7–1.0 per cent at 200 days. Calculations from Teissier's data (1931) show a similar steady decline in lipid phosphorus from 0.089 per cent in 10-mg. larvae to 0.053 per cent in 205-mg. larvae, roughly corresponding to from 2.34 down to 1.39 per cent in values for phospholipid. Needham (1942) summarized earlier work on phosphatides in the chick by stating that they are built into the embryo bradyauxetically with the relative growth constant at about 0.75. This value agrees fairly well with that found for meal worms (Table 8, B). In mammals the picture is confused by the rapidly changing water content immediately after birth. In terms of dry weight, however, both Sinclair (1930) and Williams *et al.* (1945) found decreasing percentages of phospholipid in the postnatal development of the rat. Boyd (1935), on the other hand, found an increase in the wet-weight concentration of phospholipid in fetal rabbits with rates presumably varying with the degree of physiological activity (i.e., growth of the tissues), while Gortner (1945) reported practically no change in fetal pigs during development.

The total cholesterol in meal worms at various ages was found to be quite constant in terms of percentage of wet weight. This is in agreement with the concept of the constancy of cholesterol levels in many tissues (Bloor, 1945). Unvarying levels were also found by Gort-

ner in pig fetuses, although again Boyd found a substantial rise in the free cholesterol paralleling that of the phospholipids in fetal rabbits.

Needham (1942) pointed out that, when the magnitudes of various chemical components are plotted against the magnitude of the entire organism, the ratios obtained constitute a type of "chemical ground plan of animal growth." In terms of heterauxesis, certain of the relationships examined here for meal worm larvae show clear-cut results. The relative rate of increase of total lipid with respect to the total wet weight was found to be consistent with that found by Teissier and to be significantly tachyauxetic. The constants for neutral fat and for total fatty acids against total lipids similarly were found to be significantly greater than 1.00, while that for phospholipid against total lipid was found to be bradyauxetic. It is possible to express the relation of each of these lipid entities to the total wet weight by multiplying the heterauxetic constants of Table 8 by 1.149, which is the k -value for total lipid versus the entire organism. Similarly, these relationships can be expressed in terms of total dry weight of the larva by further dividing by 1.06, which is the value for k found by Teissier for the relation between dry weight and wet weight. The constants arrived at by these calculations, which are given in Table 9, represent a type of ground plan of lipid growth in meal worm larvae expressed in terms of the concepts of chemical heterauxesis.

Interesting results are obtained when data from Series II at 225 and 250 days at 25° and at 225, 250, and 275 days at 30° C. are combined with the material up to 200 days already presented. When these additional points are included, the k -value for total lipid verses the entire organism at 25° is 1.210 and at 30° it is 1.054, in contrast to the values of 1.202

and 1.165, respectively, given in Table 5 for the coefficients of all the data at each temperature. For all the temperatures together, the regression coefficient becomes 1.135, in comparison with 1.149, which is obtained when the data are restricted to 200 days. The significance of these calculations lies in their demonstration that, when the total weight of the organisms declined, the total lipid decreased proportionately, so that a relatively fixed pattern was maintained.

The question of the flexibility or deformability of such a pattern under externally imposed stresses is part of the larger problem of the dissociability of ontogenetic mechanisms in general. Needham (1942) has shown that in many respects the various processes of growth, development, and metabolism can occur independently. Growth and differentiation, nuclear and cell division, histogenesis and organogenesis, and growth and metabolism have been shown to be differently related to one another, depending upon the conditions under which they are examined. Whether temperature has any effect in disengaging any of these mechanisms reduces in large part to the role of mere changes in the velocity at which these events occur.

Various aspects of lipid heterauxesis in meal worms have been seen here, by various analyses, to be independent of temperature. Not only did the relative growth constants, by and large, fail to show any significant differences on direct comparison, but more elaborate evaluation by analysis of variance techniques showed that changes in temperature were likely to be less important than variation from experiment to experiment. This finding is consistent with the data collected by Needham, which point to the conclusion that chemical composition is related to size and weight rather than to age.

The meal worms in the present study were subjected not only to different temperatures but at the same time to a differing set of humidities and vapor-pressure deficits. This combination of circumstances and the growth curves which they produced served to emphasize the subtle effects which result from simple ecological factors operating together. That the differential growth ratios remained constant in spite of the differences in temperature and degree of moisture in the environment serves to under-

TABLE 9

HETERAUXETIC CONSTANTS IN TERMS OF
WET WEIGHT AND DRY WEIGHT

Component	Total Lipid	Wet Weight	Dry Weight
Dry weight.....		1.06*	
Total lipid.....		1.149	1.084
Neutral fat.....	1.066	1.224	1.154
Total fatty acids..	1.019	1.171	1.104
Phospholipid.....	0.628	0.722	0.681
Cholesterol.....	0.752	0.863	0.814

* From Teissier, 1931.

line the conclusion that the magnitude of the organism, rather than the curve of its development, is the independent variable of chemical heterauxesis.

SUMMARY

1. Larvae of the meal worm (*T. molitor* L.) were raised at 20°, 25°, and 30° C. The cultures were sampled periodically and were analyzed for total fatty acids, cholesterol, and phospholipids. Total lipid and neutral fat were calculated from these determinations.

2. The dependence of rate of growth and duration of larval life on humidity, as well as on temperature, was confirmed.

3. Total lipid, neutral fat, and total fatty acids became increasing proportions of the wet weight as larval growth progressed. Phospholipid declined very slowly, while total cholesterol remained

practically constant in relation to wet weight.

4. Neutral fat increased, while phospholipid decreased in relation to total lipid as the larvae developed; total cholesterol decreased very slowly.

5. Examination of the data in terms of chemical heterauxesis revealed that total lipid is significantly tachyauxetic in relation to wet weight. With respect to total lipid, neutral fat and total fatty acids are tachyauxetic, while phospholipid and total cholesterol are bradyauxetic; these relationships are statistically significant.

6. At the three temperatures studied, the heterauxetic constants for each component do not differ significantly either by direct comparison or by analysis of variance.

7. The trends in lipid composition during the development of *Tenebrio* larvae are consistent with those known to exist in other insect larvae and in vertebrate embryos.

8. The heterauxetic constants represent a type of chemical ground plan of larval growth. This pattern appears to be unchanged by varying external conditions of temperature and humidity.

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